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RADIOBIOLOGICAL BASIS FOR THE WHOLE BODY RADIATION SYNDROME*

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Mister President and colleagues it is indeed a great honor and pleasure for me to have been invited to present this material before the German Roentgen Society. When our German colleagues visit us in the United States, they have been considerate enough to speak in English. Conversely, I shall attempt to read my contribution in German. I only hope that when I have finished you will not look at each other and say "Well at least I have learned one thing, never before did I realize how much the English language sounds like German."

And now with your indulgence I shall read my paper.

RADIOBIOLOGICAL BASIS FOR THE WHOLE BODY RADIATION SYNDROME

It is of course well-known that whole body radiation profoundly affects cell populations in the hematopoietic system. This occurs in all species; however there are marked species differences in sensitivity and in the time course of events. This is illustrated in the first slide, and may I now have the first slide please.

Slide 1

Here we see the neutrophilic granulocytes on the ordinate plotted against days after radiation on the abscissa, for three species. The dose levels are in the same range, around 350 r. Note that the rat is little effected at this dose level; the dog and man are profoundly effected. Note also the marked difference in the time course of events, with changes occurring quite early in the rat, later in the dog, and still later in man. Slide off please.

It is my purpose now to present experimental data, of our own and from the literature, to examine the degree to which the profound and potentially lethal effects on the hematopoietic tissues can be explained on the basis of known effects of radiation on involed hematopoietic cells themselves. Or alternatively is it necessary to invoke other, perhaps more generalized systemic changes, that indirectly or secondarily result in the profound marrow aplasia and pancytopenia that is seen. I shall also advance a tentative hypothesis to explain the marked species difference in sensitivity, and the marked difference in the time course of events seen in different species. In general, the radiation dose levels referred to fall roughly in the range in which clinical manifestations are chiefly the result of bone marrow damage.

I shall not be dealing with higher dose levels in which damage to the bowel epithelium, or even to the central nervous system, may lead principally to the symptomatology seen. Also, because of the short time available, I shall discuss principally the myelocytic series. Damage to this system correlates well with mortality rate, although damage to other cell series are obviously of importance also. Hence, unless stated otherwise, I shall be speaking of the neutrophilic series.

Before proceeding, it is necessary to recall the general nature of any proliferating system such as the bone marrow. A model for this system can be drawn as complicated as one wishes. In Slide 2 is shown a diagram indicating a highly over-simplified model, showing the compartments that must be present.

Slide 2

The slide is rather self-explanatory. A stem cell must exist. The cell cannot only produce daughters that are capable only of maturation and/or further division with ultimate function and death, but also cells which are capable of producing daughters that are stem cells. The morphological identity of these stem cells in not known with certainty. Nor are their relative numbers known, although they must constitute a fraction of one per cent of the total marrow cells. Division of the stem cells to produce more stem cells can be termed (quotes) "vertical" (end quotes) division. That which produces maturating cells can be called (quotes) "horizontal" (end quotes) divisions. In the dividing and maturating compartments of the marrow, one would expect many more mature than immature cells. In the simplest concept one would expect the relative numbers to increase approximately exponentially with degree of maturity. It is not known how many divisions are undergone by a cell in the entire

maturating process. Nor is it known accurately, the total time from which a cell becomes recognizable as a myelocytic precursor, until it maturates, becomes functional and dies. An estimate is shown at the bottom of the slide however and it is probable that this time is approximately three to three and one half days for the rodent; perhaps five to six days for the dog and man. Slide off please.

Let us now turn to the effects of radiation on these various compartments in the hemopoietic system. Significant effects on the non-dividing maturating and functional cells in the marrow and blood can be dismissed at once. Doses required for direct damage to these myelocytic cells are far beyond the range of biological interest here. Let us then deal first with the effects of radiation on the stem cell compartment, and then on the dividing and maturating cells of the marrow.

First, then with regard to the stem cell compartment. Considerable direct information is available with respect to their sensitivity, at least in the mouse. And one can deduce additional information from so-called (quotes) "survival" (end quotes) curves that have been obtained -- first for mammalian cells grown in culture, and later for a variety of systems. Such a survival curve for mouse stem cells, taken from the work of Till and McCullough, is shown in the next slide. May I have the next slide please.

Slide 3

In these studies, bone marrow was irradiated either in vitro or in vivo. The irradiated marrow was then injected into heavily irradiated recipient mice, and the survival of stem cells in the irradiated injected marrow was evaluated in terms of ability to produce visible nodules or colonies of cells in the spleen of the heavily irradiated recipient mouse.

The general nature in the slope of this curve is the same as that obtained for HeLa cells in culture and for a variety of other proliferating cell types in other systems. Before remarking further on this curve specifically, let me make a few remarks relating to the general nature and meaning of all such curves so far obtained.

First, these are termed (quotes) "survival" (end quotes) curves, but the meaning of survival is special. Survival means that the cell and its progeny have been able to continue to divide long enough so that an entire clone has developed. With HeLa cells, these are visible colonies on the arter (?) plate. With stem cells, it is a visible colony in the spleen. Thus the time for observation, or scoring, is several days to weeks. Thus survival does not mean life or death of the cell as determined by morphologic damage to the directly radiated cell, which might be visible on the microscope after some few hours or days. Whether survival is a measure of the proliferative capacity of the irradiated cell -- the capacity for sustained proliferation of it and its progeny.

Second, by this criterion of survival, note how radiosensitive such proliferative mammalian cells are. Actual dose-effect relationships vary with type of cell. But uniformly it has required only of the order of fifty to one hundred fifty r to reduce the number of viable cells to one half. This is in contrast to morphological criteria. For most cell types, an extremely large dose of radiation is required to produce detectible damage before the first division of the cell. Note also the exponential nature of the curve. This means that <u>some</u> viable cells remain even after very high doses.

Third, it is important to realize the course of events between the time of exposure, and that of scoring, several days later. Many cells have shown chromosomal or other cytological damage, and the number that shows such damage increases greatly with increasing dose. Presumably the proliferative capacity of a cell, as indicated by colony size after a period of time can be curtailed with visible evidence of damage to the original cell actually radiated. Thus the quality as well as quantity of surviving cells is affected. The more severely injured may not survive one division. The less severely injured may go through one, two, three or even more division, before all progeny dies. Only completely non-injured cells, or slightly injured cells will survive to produce a sustained clone of cells. However, there is good evidence from HeLa cell studies that even the (quote) "survivors" (end quote) may have a reduced proliferative potential, perhaps related to a prolonged generation time. With increasing dose, the colonies that survive are smaller.

We might then expect, that when a population of proliferating stem cells in the marrow is irradiated, that similar events would occur. At increasing, but still relatively low doses, few cells would be able to proliferate at all, and many of these for only a few division. At higher doses, most would not survive even one division, and only the relatively intact or completely intact cells would remain to replenish the population.

We may now return specifically to the curve shown here in Slide 2 for the mouse. This almost certainly is for stem cells in the bone marrow, and each colony counted in the spleen of the heavily irradiated daughter represents probably a single surviving stem cell in the

injected marrow. Note that the LD_{50}, or the dose to kill half of the cells is only about seventy-five rads. This means that at the LD_{50} for the mouse or rat, around seven hundred and fifty rads, only about zero point one per cent, or one out of every one thousand stem cells remains relatively intact or completely intact. At lower doses, less severely injured cells would proliferate for a few divisions, but would be incapable of sustaining the population or producing a visible nodule. That is to say, some irradiated stem cells divide only twice some only three times etcetera before all progeny die. Slide off please.

Before translating the above into what might be expected to been in the marrow and peripheral blood in the mammal given whole body irradiation, it is necessary to consider the effects of radiation on the dividing and maturating components of the marrow. The phenomena are similar to those seen with tissue culture and other similar self-sustaining populatings, but to a lesser degree. We have some pertinent information on myelopoiesis in the rat; however it will be necessary to bring in some data on the erythrocytic series as well in order to make the desired points.

First, grossly abnormal cells are seen easily at high dose levels and can be seen with little difficulty at lower dose levels. May I have the next slide please.

Slide 4

Here is shown the number of mitotically connected abnormalities in the myelocytic series, arbitrarily scored, versus time after irradiation. The mitotically connected abnormalities are very easily seen, and do not appear at all until some of the irradiated cells have had time to go through mitosis and divide. The abnormalities are

easily visible, as chromosome fragments, fragmented nuclei, giant cells, binucleated cells, etcetera. Most of the abnormal cells at these high dose levels were sufficiently mature and appeared early enough, so that the damage seen must represent damage in the maturating dividing compartments, and not only in the stem cells. Note that the number of abnormal cells increased with increasing time but that the abnormal cells remained for a shorter time at the higher dose levels. In all cases, the abnormal cells were (quote) "swept out" (end quote) rapidly, and had disappeared within the total time required for the earliest recognizable precursor to differentiate and leave the marrow. Thus there seems to be no delay in maturation of these abnormal cells, and they either leave the marrow to the blood stream or are quickly removed if they disintegrate. Slide off please.

But what about the remaining cells that show no detectable injury? Here we have some information, on both the most mature myelocytic cells and on the erythrocytic cells. First, with respect to the myelocytic cells. If a single injection of tritiated thymidine is given to a normal rat, the labeled granulocytes appear in the peripheral blood starting at about three and one-half days after thymidine administration. After doses of _____ rads, the labeled cells appear at approximately the same time. This indicates no significant degree of delay in maturation of these cells, resulting from the exposure. The time for maturation from the last DNA synthesis, through the last division, and through the final maturation and release to the blood is not altered. Similarly for the erythrocytic series, and may I have the next slide please.

Slide 5

This represents the progression in time of tritiated thymidine labelled cells, from stages that normally lable at once or immediately after tritiated thymidine injection into normally non-labeling more stages. The slide shows the rate of progression in the normal, and in a dog given a one hundred fifty r whole body irradiation. From these data it would seem clear that although maturating dividing cells are injured to varying degrees, depending on dose, the remaining cells continue to maturate and leave the marrow at essentially the normal rate. Lights please.

With the above discussion of radiation effects on both stem cells and maturating-dividing cells, one can then anticipate what would happen in the irradiated marrow and see if the predictions agree with available data. It is easier here initially to look at higher dose levels in the high lethal range or somewhat higher. Here no survival occurs normally and we are dealing with the so-called degenerative phase of bone marrow damage only. Here quantitative data on numbers of cells, not obtainable with smears alone, are required for evaluation. Such data are available only for the rodent as shown on the next slide. Next slide please.

Slide 6

Here is shown a plot of the total number of cells in the bone marrow of the rat femur, versus days after eight hundred r whole body irradiation. this represents the relative number of myelopoietic cells of all stages of maturation. Note that the curve has a (quote) "shoulder" (end quote), perhaps reflecting the time required formany of the cells to pass through stages of the cell cycle preceding mitosis. The curve then

falls exponentially, as would be expected, and the low point is reached -- also as expected -- at three to three and a half days. Note also beginning regeneration, even though the animals die.

With the marrow (quote) "emptied" (end quote) of myelocytic precursors by three and one half days, and with the known very short residence time of granulocytes in the peripheral blood, one would expect the blood to be depleted shortly after the marrow reaches a low point. May I have the next slide please.

Slide 7

Here are shown the curves for the relative number of granulocytes in the peripheral blood versus time. Curves are shown for three species, the dog, the rat and man, and the doses are of the same order of magnitude but high -- around eight hundred to a thousand r. It will be noted that granulocytes of the rat reach a low just after the myelopoietic elements in the bone marrow reached a minimum. The somewhat earlier decrease for the dog is probably due to the fact that the dose of radiation was higher than for the rat. The human data are complicated by the fact that the radiations were mixed -- neutrons, gamma and X-rays -- and that the dose to the tissues was markedly non-uniform. Nonetheless, the granulocytes were depleted by day five to six. Slide off please.

Thus, although there exist differences of a day or two depending on species and dose, the general pattern of degeneration at high dose levels seems clear. The sequence of events is as though the numbers of stem cells were rather suddenly reduced drastically in number. Any remaining stemm must repopulate their own kind, as well as attempt to feed cells into the maturating pool. The cells that were irradiated while maturating either

die or maturate at essentially the normal rate, and, with little or no replacement from the stem cells, the marrow is completely devoid of formed precursor elements in the expected time of three to five days. In the peripheral blood, this is reflected in essentially total depletion of peripheral neutrophiles by day four to six. The individual dies a few days after granulocyte depletion. The exact time depends in part on the time and nature of the infection that is certain to develop, and part on when the peripheral platelets are also depleted, and severe hemorrhage complicates the picture.

We may now proceed to the regenerative phase, and it is initially easier to deal with relatively high radiation dose levels. Here spontaneous regeneration is possible for the rodent, but not for the dog or for man. May I have the next slide please.

Slide 8

Here we see the blood granulocyte count plotted against days after whole body exposure, for the dog and the rat. At the higher dose levels, spontaneous regeneration is possible for the dog. However ~~xxxxxxxxx~~ regeneration occurs in dog or man only if normal marrow, that is to say normal stem cells are injected after radiation. In the rat, it is seen clearly that regeneration begins early -- as early as the fourth or fifth day. But is also seen that this regeneration is short-lived -- it has been termed (quotes) "abortive regeneration" (end quote), and may well be reflected in the marrow. At least in the rat, on day five or six are seen small islands of (quote) "regeneration" (end quote), of cells that can be identified as myelocytic. However many of the cells appear to be abnormal and the colonies are reduced in size or appear

to have disappeared one to two days later, when the cells have had a chance to divide a few more times. This could be interpreted that only the colonies derived from relatively unaffected or completely unaffected cells can produce sustained colonies, and it is these relatively unaffected cells that proceed to the true and sustained regeneration seen later. This permanent regeneration is reflected in the relatively smooth granulocyte curve seen beyond day twelve.

Now let us look at the curve for the dog at one thousand r. At this dose death is certain in eight to twelve days, even if replacement measures such as platelets, fluids and antibiotics are used. However, if an intravenous infusion of the dogs own bone marrow is given, the marrow regenerates with remarkable rapidity as reflected in the curve on the slide, and the animal survives. Apparently enough normal stem cells have been added to the surviving relatively intact ones, such that regeneration can proceed sufficiently far and fast to prevent death. At the far right in the slide, for comparison, is given the regenerative curve for a dog exposed to a much lower dose. The dose was two hundred and fifty r, which is still lethal for about half of a population of animals so exposed. Slide off please.

Several conclusions seem reasonable from these comparative data. The dog can survive at high doses as does the rat, with rapid marrow regeneration as in the rat. However, for such regeneration, it is necessary that additional normal stem cells must be supplied. Thus survival depends on the number and quality of stem cells present. The curves, beyond the region of (quote) "abortive rise" (end quote) for the rat, appear identical for both the rat and the marrow treated dog. Thus the curve for the dog may reflect the

(quote) "true" (end quote) regenerative clones in both species. This would indicate that the dog and rat act differently with respect to lethal dose and time course of events after exposure, because of differential sensitivity of the stem cell population, or the ability of this irradiated population to feed into the maturating cell groups. At six hundred or seven hundred r, the rat has remaining stem cells in sufficient quantity, and of such quality that regeneration is possible before peripheral blood depletion leads to death. The dog at this high dose has so few intact stem cells that adequate regeneration is not possible before death ensues. The fact that the dog marrow regenerates rapidly after bone marrow infusion, at doses of radiation four to five times the otherwise lethal dose, indicates that it is not sustained stromal damage to the marrow, or some general stomach disturbance resulting in continued marrow aplasia, or a suppressing (quote) "feedback" (end quote) from the spleen or other organ that is responsible for prolonged marrow failure and delayed regeneration at low doses in the dog and man. Rather, it is much more likely to be qualitative and quantitative differences in effects of radiation on the stem cell population.

The fact that the curve for regneration for the untreated dog, at one-fourth the radiation dose as seen on the slide, is considerably more shallow and delayed than for the lethal receiving a much higher radiation dose, plus marrow. This can be taken as evidence that the remaining irradiated stem cells are not only reduced in quantity, but in quality as well. Or alternatively, it could reflect simply a greater number of fully viable stem cells in the marrow-treated animal. Further evidence for qualitative defects to explain this phenomenon will be shown in the last two slides. Next slide please.

Slide 9

Here we see the number of mitotically-connected abnormalities in the myelocytic series, versus time, in the bone marrow of man exposed in the low lethal or very high sublethal range, or a dose of approximately three hundred r. Note that while such abnormalities have disappeared completely from the rat bone marrow by day three or four at the latest, in the high dose levels, in the human being they persist at least until day nine and likely well beyond. This is much too long for the original damage to have occurred in the maturating-dividing pool, and this undoubtedly reflects damage to the stem cells. It is also of considerable importance to note that while the abnormalities do persist for many days, they do disappear, or at least essentially all of them disappear. This would indicate further that some damaged stem cells can divide several times to produce abnormal progeny, but that eventually the damaged stem cell and all of its progeny die and are removed from the marrow. Further evidence can be deduced from the next slide, and if I may have this now please.

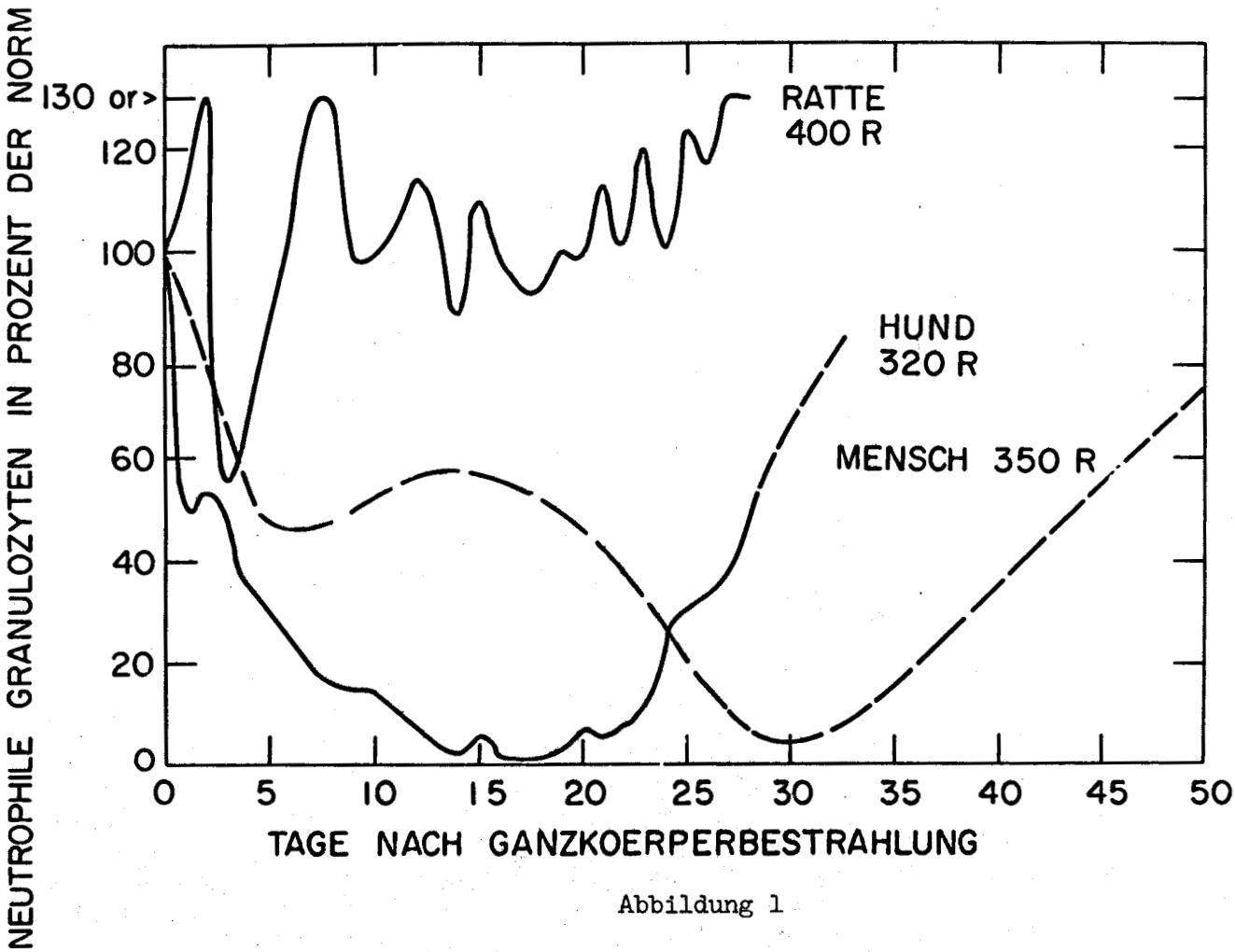
Slide 10

This slide you will recognize as the same as the first slide I showed. Here we see the granulocyte count versus time in the peripheral blood of the rat, dog and man exposed to doses of radiation around three hundred and fifty r. These doses are in the lethal range for dog and man, but are well below the lethal dose for the rat. Note first the curve for the rat. There is an initial dip; however regeneration is early and marked. The count is normal or above normal by day six. However, a markedly different situation exists for the dog and man,

as is evident from the curves. The curves for both species, as opposed to that for the rat, in time reach essentially zero. However, the time required for the counts to fall to zero is quite long in the dog and man as compared to the rat. If this eventual marked fall were due merely to killing of stem cells, or killing or injuring maturating-dividing cells, then the low point would have occurred no later than day five or six for both species. Further, from the data on marrow injection in the dog, marked regeneration of stem cells, had they been normal, would have been well underway by day twelve to fourteen. This evidence again points to damaged stem cells as the explanation for the delayed fall in count, and the delayed pattern of regeneration seen in dog and man as opposed to the rat. At these relatively low doses, the number of completely viable stem cells is reduced to considerably lower levels than in the rat. In addition, however, a number of damaged stem cells remain. These produce damaged offspring that can survive for several generations, but they and all their progeny die out in a matter of days to weeks. It is at this time that the low point in the curve is reached. A rise in the curve occurs in survivors, when the very few initially remaining stem cells have replenished their own number to a degree, and are able to feed cells into the differentiating pool. May I have the lights please.

In summary, I have presented data showing a marked species difference in the degree of damage, and in the course of events seen in the bone marrow and peripheral blood after a given dose of radiation. The data taken together indicate strongly but do not prove, that the picture seen results primarily from damage to the stem cell population. This damage to the

stem cells is manifested later in the more mature differentiating cells of the marrow, and in the peripheral blood. The picture seen results both from death of stem cells, and also injury leading to reduced proliferative capacity, with later death of the injured stem cell and all of its progeny. Thus the picture seen is a result of both quantitative and qualitative changes resulting directly from the exposure of the stem cells, and species differences relative to sensitivity and time course of events appear to depend to a large degree on differential sensitivity of the bone marrow stem cell populations to death and injury.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100
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RATTE
400 R
HUND
320 R
MENSCH 350 R
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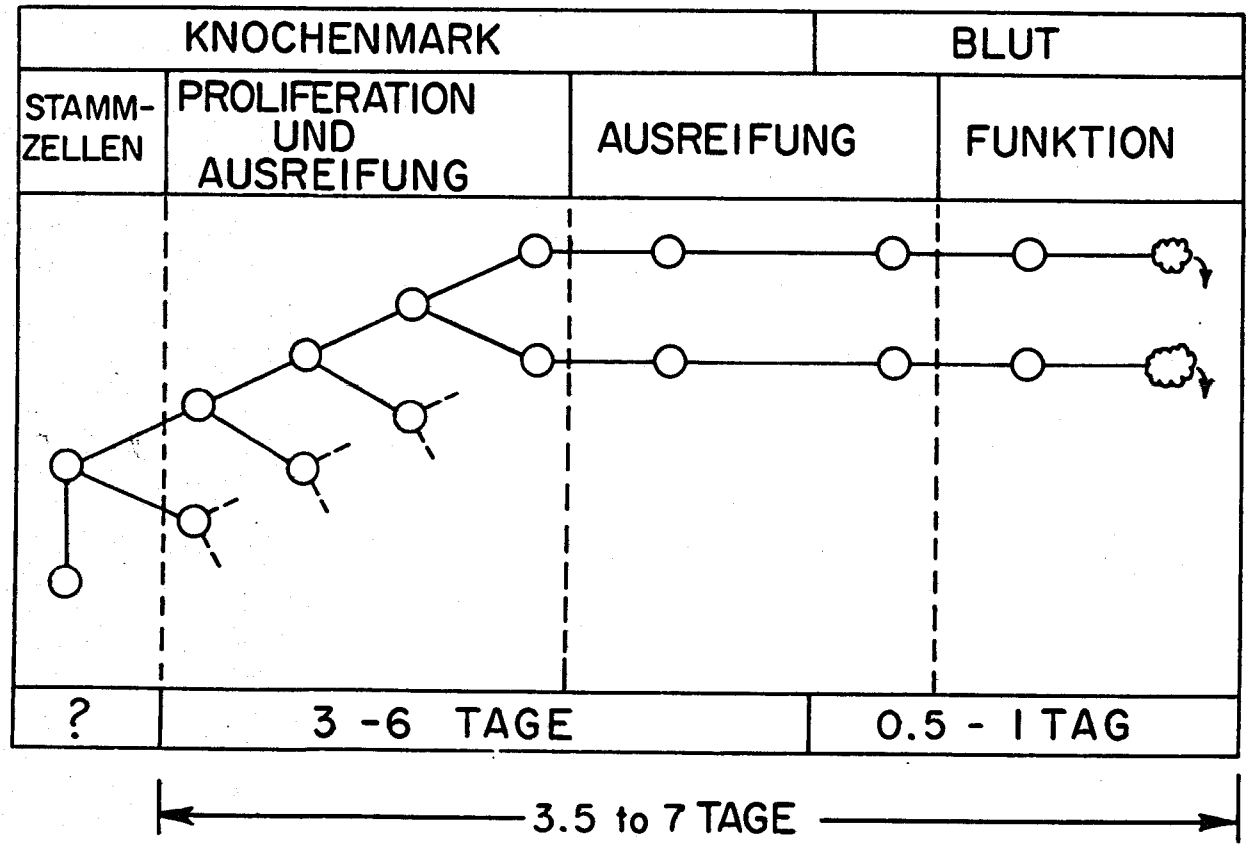
TAGE NACH GANZKOERPERBESTRAHLUNG

Abbildung 1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BLUT
STAMM-ZELLEN
PROLIFERATION UND AUSREIFUNG
AUSREIFUNG
FUNKTION
?
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0.5 - I TAG
3.5 to 7 TAGE

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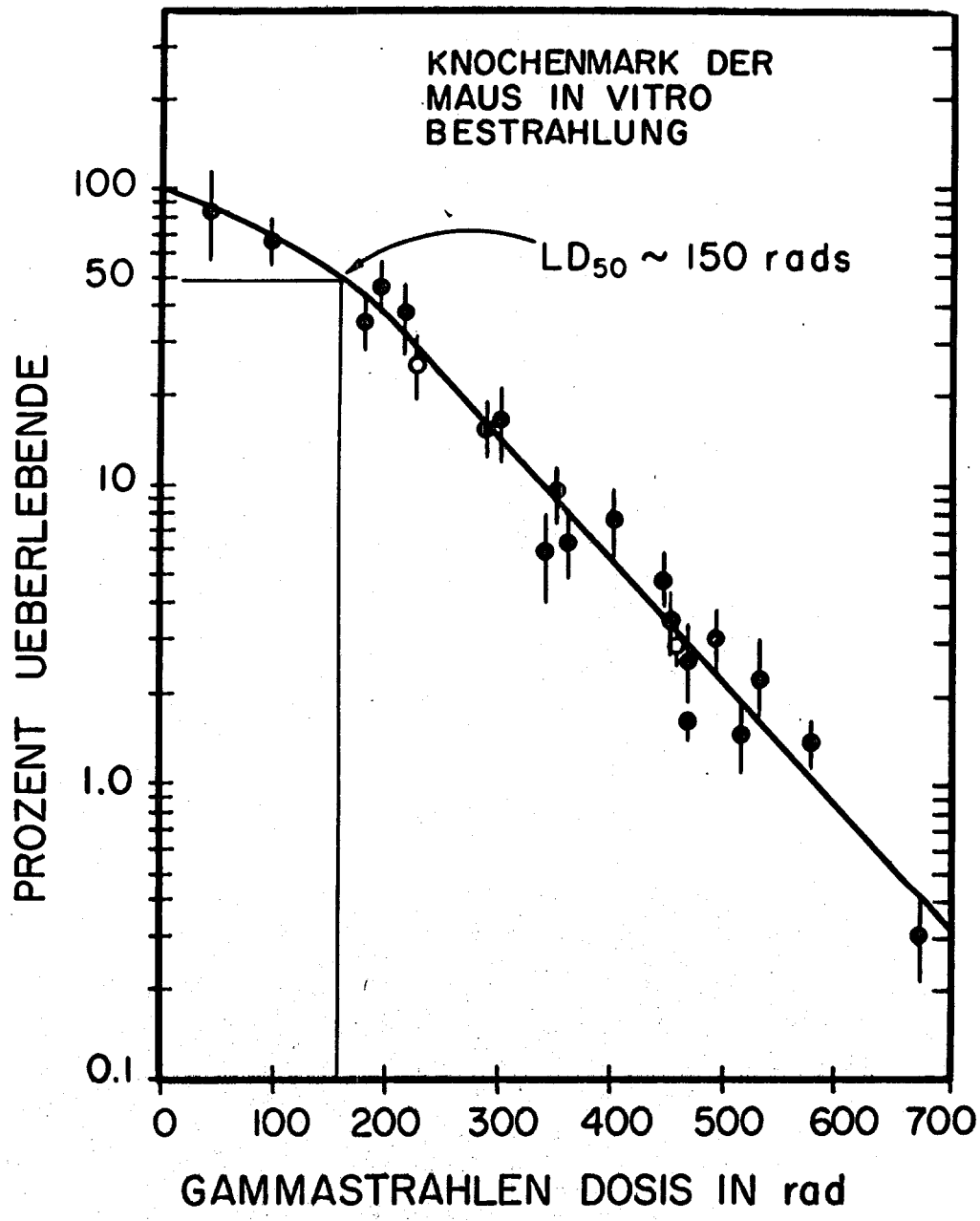
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MAUS IN VITRO
BESTRAHLUNG
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PROZENT UEBERLEBENDE
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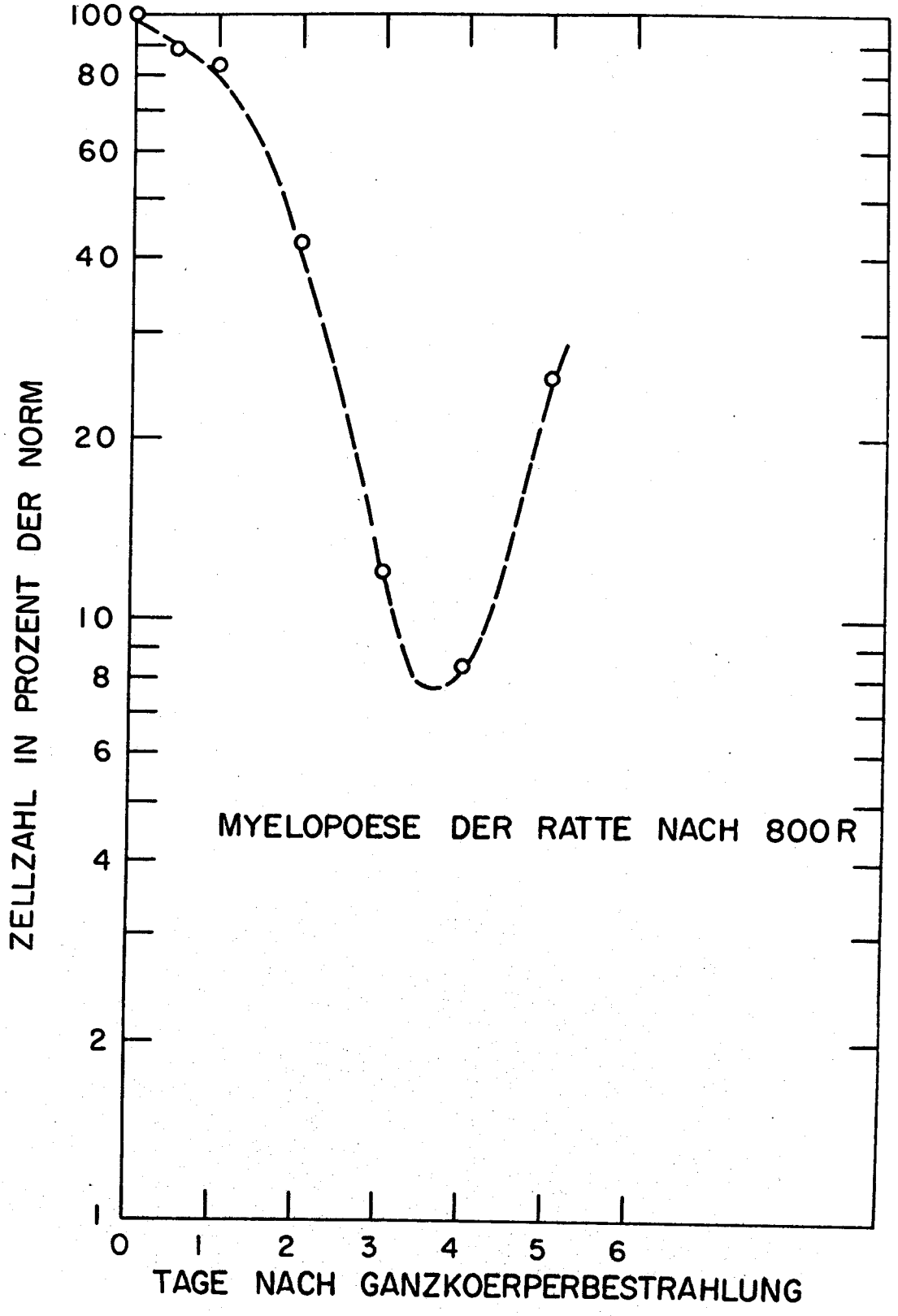
600
700
GAMMASTRAHLEN DOSIS IN rad

Abbildung 3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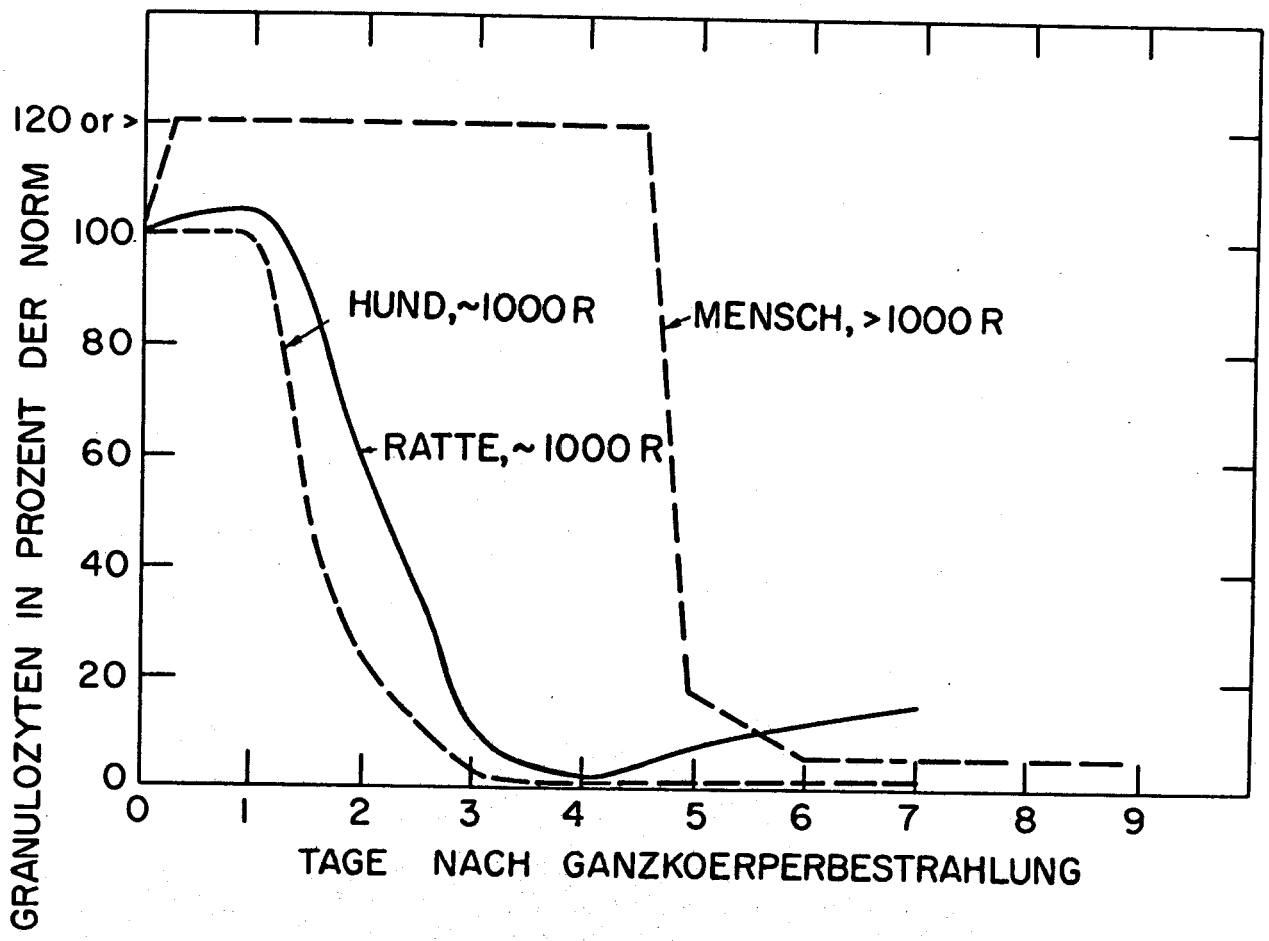
TAGE NACH GANZKOERPERBESTRAHLUNG

Abbildung 4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GRANULOZYTEN IN PROZENT DER NORM
HUND,~1000 R
MENSCH, >1000 R
RATTE,~1000 R
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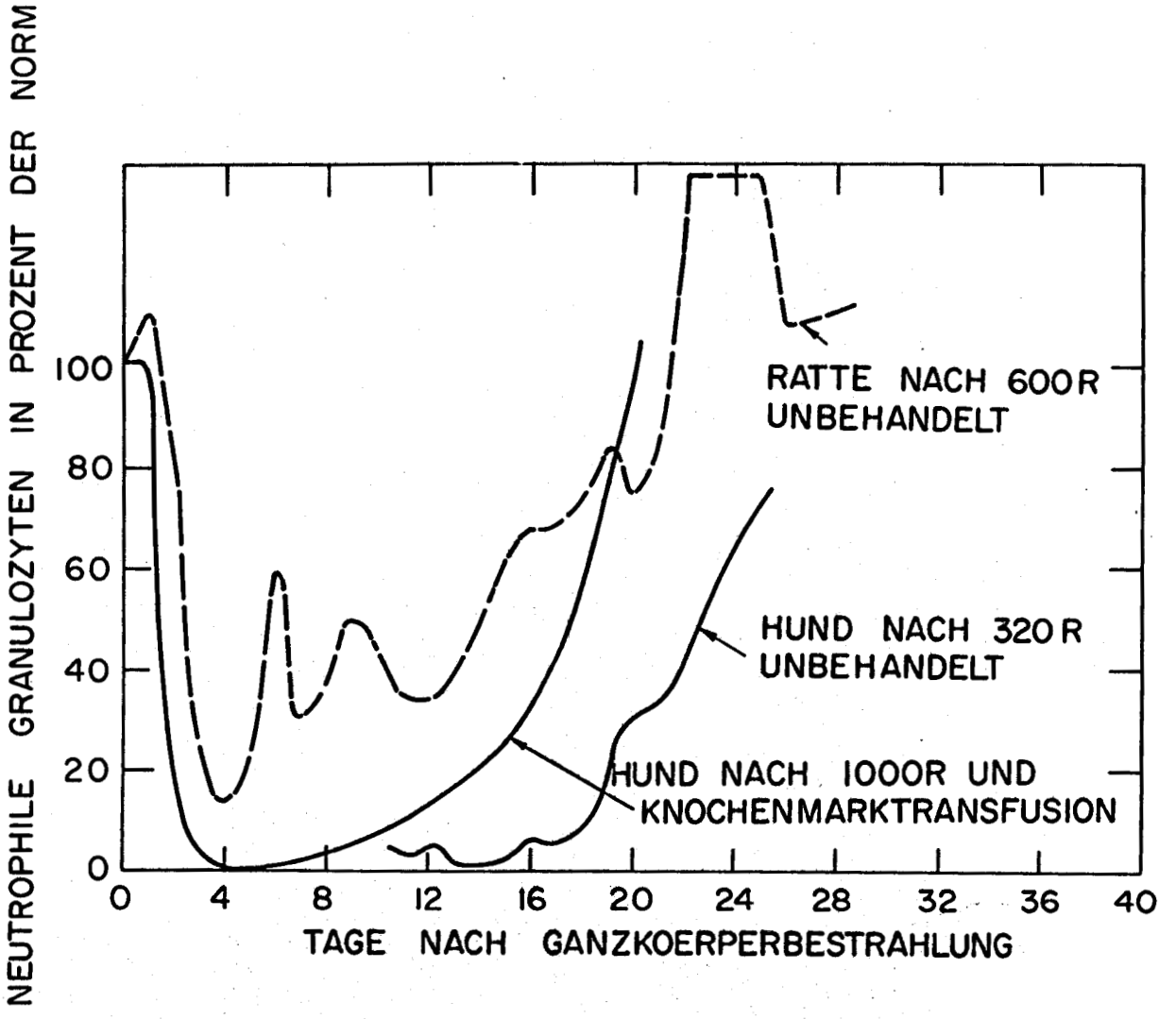
TAGE NACH GANZKOERPERBESTRAHLUNG

Abbildung 5


NEUTROPHILE GRANULOZYTEN IN PROZENT DER NORM
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TAGE NACH GANZKOERPERBESTRAHLUNG
RATTE NACH 600R
UNBEHANDELT
HUND NACH 320R
UNBEHANDELT
HUND NACH 1000R UND
KNOCHENMARKTRANSFUSION

Abbildung 6